

# Effect of ascorbic acid on apparent iron absorption by women with low iron stores<sup>1-4</sup>

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**ABSTRACT** The effect of ascorbic acid supplementation on apparent iron absorption was tested in women with low iron stores. For 10 wk, 25 healthy nonpregnant women, aged 20–45 y with low serum ferritin (3.5–17.7  $\mu\text{g/L}$ ), consumed either a diet with predicted poorly bioavailable iron or a typical Western diet, classified according to dietary meat and ascorbic acid contents. Meals were supplemented with ascorbic acid (500 mg, three times a day) for 5 of the 10 wk, in a double-blind, crossover design. Ascorbic acid did not affect most biochemical indexes of iron status, the biological half-life of  $^{59}\text{Fe}$ , or apparent iron absorption (diet – feces) from either diet, but slightly increased serum ferritin (11.9 vs 10.7  $\mu\text{g/L}$ ,  $P < 0.06$ ) when data from both diets were combined. These results support other evidence that ascorbic acid has less effect on iron bioavailability than has been predicted from tests with single meals. *Am J Clin Nutr* 1994;59:1381–5

**KEY WORDS** Iron bioavailability, absorption, ascorbic acid, ferritin, thyroid hormones

## Introduction

Ascorbic acid consistently enhances absorption of nonheme iron from single meals, as indicated by erythrocyte incorporation of isotopic tracer (1, 2). However, ascorbic acid supplementation (2 g/d) with meals consumed by volunteers on self-selected diets for 16 wk did not affect body iron stores as indicated by serum ferritin (3). Failure to influence body iron stores was not explained by adaptation to the ascorbic acid; enhancement of iron absorption from single test meals by ascorbic acid was demonstrated at the beginning and end of the 16-wk experiment (3). In a similar report, supplementation with 100 mg ascorbic acid three times per day with meals for 8 wk did not affect serum ferritin of volunteers on self-selected diets (4). Subsequently, a 9-mo study of menstruating women indicated no effect of ascorbic acid supplementation of meals (100 mg given three times per day, compared with other subjects receiving 250 mg at night) on serum iron, total iron-binding capacity, transferrin saturation, or serum ferritin (5). The subjects in these studies may have been consuming diets with substantial amounts of meat, and thus their diets may have already promoted nonheme-iron absorption without ascorbic acid supplementation. These results raised doubts about the effectiveness of ascorbic acid for enhancing iron bioavailability from common diets for an extended time.

For 5 wk, ascorbic acid improved apparent iron absorption (balance method) in women who had been depleted of iron by

diet and phlebotomy, and who consumed a diet predicted to have poorly bioavailable iron (6). This study demonstrated that ascorbic acid could improve apparent iron absorption over several weeks. However, two related questions remained unanswered: 1) In population groups with low iron status (not depleted by phlebotomy), can iron absorption be substantially improved by ascorbic acid (with or without a change in serum ferritin)? and 2) Can this occur in populations with a normal Western diet containing meat and ascorbic acid, or only in individuals who consume diets with poorly bioavailable iron?

The objective of this investigation was to determine whether ascorbic acid would improve apparent iron absorption by women with low iron stores who were fed either a diet with predicted poorly bioavailable iron or a typical Western diet. Because differences in thyroid hormones (7) have been associated with iron deficiency, we also tested the effect of ascorbic acid supplementation on circulating thyroid hormones in these women with low iron stores.

## Methods

### Protocol

For 10 wk, volunteers consumed either a diet with predicted poorly bioavailable iron (experiment 1,  $n = 13$ ) or a typical Western diet (experiment 2,  $n = 12$ ). The two experiments used separate, independent groups of volunteers. Meals were supplemented with ascorbic acid (500 mg three times per day) for 5 of the 10 wk in a double-blind, crossover design. Apparent iron absorption (diet – feces) was determined for 9 d, and 40 mL blood was collected the last day of each 5-wk dietary treatment. Oral  $^{59}\text{Fe}$  was administered at the beginning of the 10-wk study

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<sup>3</sup> Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the US Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

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and monitored by whole-body scintillation counting to detect possible changes in biological half-life associated with ascorbic acid supplementation.

### Subjects

Volunteers were recruited through public advertising and selected on the basis of interviews and a blood test to assess iron status and general health. Twenty-five healthy, nonpregnant women aged 20–45 y ( $\bar{x} \pm \text{SD}$ :  $166 \pm 6$  cm tall, weighing  $67 \pm 14$  kg) with low serum ferritin ( $3.5\text{--}17.7 \mu\text{g/L}$ ) participated with informed consent. This study was approved for human subjects by the US Department of Agriculture Human Studies Review Committee and by the University of North Dakota's Radioactive Drug Research Committee and Institutional Review Board. The volunteers had not donated blood or taken iron supplements providing  $\geq 20$  mg/d for the previous 6 mo. Five individuals who reported taking iron supplements providing  $< 20$  mg/d were asked to discontinue these as soon as their application was received ( $\geq 2$  mo before the beginning of the study); 20 individuals reported taking no iron supplements.

### Diets

Participants lived in their own homes and obtained all of their food from the research center. On weekdays they ate one meal daily (usually breakfast) at the research center. Other food was carried out in insulated containers and heated as necessary in microwave or conventional ovens at home or work sites. All foods were weighed and completely consumed, except for a few optional foods provided by the research center (eg, sugar-free soft drinks and sugar-free chewing gum). City water was consumed as desired. Tea was excluded from the diet. Coffee consumption was individualized, allowing up to two cups of coffee (2 g instant coffee) per day, which was constant throughout the study. The diets used a 3-d cyclic menu. Dietary energy was adjusted in 0.84-MJ (200-kcal) increments to maintain constant body weights by changing the amounts of all foods proportionally.

At an energy intake of 8.4 MJ (2000 kcal), the diet with predicted poorly bioavailable iron (experiment 1) provided 15.4 mg Fe (calculated) and 0.2 mg heme Fe [calculated (8)], and had limited amounts of meat (45 g meat/d, or 22.5 g/meal for six meals in 3 d) and ascorbic acid [59 mg ascorbic acid/d with  $< 20$  mg/meal (calculated)] (Table 1). According to the model of Monsen et al (8), predicted iron bioavailability was low for all nine major meals of the diet. The total iron consumed by the volunteers was  $16.9 \pm 1.9$  mg/d (analyzed) in experiment 1.

At an energy intake of 8.4 MJ (2000 kcal), the typical Western experimental diet (experiment 2) provided 15.2 mg Fe (calculated), 1.4 mg heme Fe [calculated (8)], and meat and ascorbic acid in amounts common in the United States [178 g meat/d, or seven portions of meat in 3 d—three as beef (100 g each), two as chicken (60 and 75 g), and two as pork (40 and 60 g)—and 99 mg ascorbic acid/d (calculated)] (Table 1). According to the model of Monsen et al (8), predicted iron bioavailability was moderate for two-thirds and high for one-third of the nine major meals of the diet. The total iron consumed by the volunteers was  $17.1 \pm 2.1$  mg/d (analyzed) in experiment 2.

Ascorbic acid supplementation was blinded to the volunteers and staff members not involved in preparation of the experimental diets. Volunteers received similarly flavored powdered ascor-

TABLE 1  
Sample menus for 1 d, providing an energy intake of 8.4 MJ (2000 kcal)

Diet with predicted poorly bioavailable iron (Experiment 1)		Typical Western diet (Experiment 2)	
Food item		Food item	
Breakfast		8	
Apple juice	95	Orange juice	115
Oat bran cereal	30	Corn flakes	25
2% Fat milk	150	2% Fat milk	150
Wheat bread	30	Wheat bread	40
Corn oil margarine	10	Butter	5
Grape jelly	20	Grape jelly	15
		Sugar	5
Lunch			
Apple juice	90	Apple juice	160
Chili		Crispy chicken	
Ground pork	22.5	Ground chicken	
Kidney beans	80	breast	75
Onion flakes	0.5	Beef bouillon	1.0
Beef bouillon	1.0	Butter	5
Chili powder	0.5	Corn flake crumbs	10
Sugar	2.0	Potatoes, canned	135
Tomato sauce	65	Carrots, canned	100
Corn oil margarine	5	Dinner roll (with refined flour)	30
Dinner roll (with refined flour)	40	Butter	10
Butter	7.5	2% Fat milk	150
Peanuts	25		
2% Fat milk	150		
Dinner			
Apricot nectar	170	Apple juice	160
Chicken-cheese casserole		Beef-cheese casserole	
Spaghetti, cooked	70	Spaghetti, cooked	55
Corn oil margarine	5	Butter	5
Onion flakes	0.5	Onion flakes	0.5
Beef bouillon	1	Beef bouillon	1
Ground chicken		Ground beef	100
breast	22.5	Cheddar cheese	20
Cheddar cheese	20	Lettuce	55
Green peas	150	French dressing	15
Angel-food cake	60	Pineapple chunks	150
		Angel-food cake	50
Snack			
Ritz crackers <sup>1</sup>	30	Ritz crackers <sup>1</sup>	20
Cheddar cheese	30	Cheddar cheese	20
Pineapple	125	Sunflower kernels	20

<sup>1</sup> Nabisco Foods, East Hanover, NJ.

bic acid supplements (0.3 g sugar-free lemonade powder, 0.5 g ascorbic acid, and 0.2 g aspartame sweetener) or placebos (0.5 g sugar-free lemonade powder and 0.5 g lactose) to mix into the beverage with each meal.

### Sample collections and chemical analyses

Excreta, diets, and blood were collected with precautions to avoid trace mineral contamination. Duplicate diets were prepared throughout each balance period. Feces were collected completely for 14 d (days 22 through 35 of the diet period). The utility of a

TABLE 2

Apparent iron absorption and clinical indexes of iron and thyroid status, as affected by ascorbic acid supplementation<sup>1</sup>

	Diet with predicted poorly bioavailable iron (Experiment 1, <i>n</i> = 13)			Typical Western diet (Experiment 2, <i>n</i> = 12)			Both diets ( <i>n</i> = 25)		
	Ascorbate	Placebo	Pooled SD	Ascorbate	Placebo	Pooled SD	Ascorbate	Placebo	Pooled SD
Dietary energy (MJ/d) <sup>2</sup>	9.53	9.34	0.36	9.70	9.70	0	9.61	9.51	0.26
Dietary iron (mg/d)	17.0	16.7	—	17.1	17.2	—	17.0	17.0	—
Apparent iron absorption (mg/d)	5.8	4.3	3.1	6.0	6.4	2.3	5.9	5.3	2.7
Hemoglobin (g/L)	132	131	4	132	130	4	132	131	4
Ferritin (μg/L)	12.9	11.4	2.5	10.6	9.7	1.9	11.9 <sup>3</sup>	10.7	2.1
Transferrin saturation (%)	22.9	24.4	2.7	23.7	21.7	1.9	23.3	23.2	2.6
Serum iron (μmol/L)	15.3	16.0	1.4	16.2 <sup>4</sup>	15.3	0.6	15.7	15.7	1.2
Zinc protoporphyrin (μmol/L)	0.31	0.33	0.06	0.31	0.34	0.05	0.31	0.33	0.05
Total T <sub>3</sub> (nmol/L)	1.9 <sup>5</sup>	1.6	0.3	1.9	1.9	0.2	1.9	1.7	0.3
Iron stores (mmol)	0.84	0.25	1.10	0.15	-0.39	1.00	0.55	-0.01	1.00
<sup>59</sup> Fe biological half-life (d)	483	444	0.67 <sup>6</sup>	—	—	—	—	—	—

<sup>1</sup> The following additional variables were not affected by ascorbate in either experiment, or with both diets combined: fecal iron, percent fecal iron, iron-binding capacity, free triiodothyronine (T<sub>3</sub>), total thyroxine, free thyroxine thyrotropin, and reverse T<sub>3</sub>.

<sup>2</sup> To convert megajoules (MJ) to kilocalories multiply by 238.9.

<sup>3</sup> Combined data suggest slightly higher ferritin concentration with ascorbic acid, *P* < 0.06.

<sup>4,5</sup> Significantly different from placebo (within group): <sup>4</sup>*P* < 0.004, <sup>5</sup>*P* < 0.02.

<sup>6</sup> Pooled SD of logarithmically transformed data; means have been converted back to original scale.

fecal marker was evaluated by analyzing appropriate fecal aliquots to compare fecal iron excretion for 9 prescheduled days (days 24 through 32 of the diet period), with iron excreted in stools designated by the visible appearance of Brilliant Blue dye (FD & C Blue no. 1; Tricon Colors, Inc, Elmwood Park, NJ) (100 mg) administered orally with breakfast at 9-d intervals (in the morning of days 24 and 33 of each diet period). Apparent iron absorption was determined as the difference between dietary and fecal iron. Iron absorption by the balance method is characterized as "apparent" in recognition of the slight underestimation of true absorption by this technique because of the inability to distinguish unabsorbed fecal iron from endogenous iron excreted in the feces. Aliquots of the diet and fecal composites were digested with concentrated nitric and 70% perchloric acids by method (II)A of the Analytical Methods Committee (9). The iron content of the digestates was determined by inductively coupled argon plasma emission spectrophotometry. Analytical accuracy was monitored through periodic analyses of certified standard reference materials from the National Institute of Standards and Technology (NIST). The analyzed values were 98.9% (*n* = 12) and 98.2% (*n* = 6) of certified values for iron in NIST bovine liver standard no. 1577a and NIST total diet standard no. 1548, respectively.

Biochemical measurements on fasting blood samples included plasma iron measured by Zeeman graphite furnace atomic absorption spectrophotometry with prior precipitation by trichloroacetic acid (10), serum ferritin measured by radioimmunoassay (Baxter Travenol Diagnostic, Inc, Cambridge, MA), percent transferrin saturation calculated from the serum iron and total iron-binding capacity (11), erythrocyte free protoporphyrin by ethyl acetate-acetic acid extraction (12), zinc protoporphyrin by hematofluorometry (13), and free and total triiodothyronine (T<sub>3</sub>), free and total thyroxine (T<sub>4</sub>), thyroid stimulating hormone (TSH), and reverse triiodothyronine (rT<sub>3</sub>) by radio immunoassay (T<sub>3</sub>, T<sub>4</sub>, and TSH assay materials from Diagnostic Products Corporation,

Los Angeles, and rT<sub>3</sub> materials from Serono, Braintree, MA). Iron stores were estimated as described by Cook et al (14).

#### <sup>59</sup>Fe retention

To determine the effect of ascorbic acid on the biological half-life of absorbed iron, 37 kBq <sup>59</sup>Fe as ferrous sulfate was consumed with orange juice at the beginning of experiment 1. Retention of the isotope was measured by whole-body scintillation counting twice weekly. Biological half-life was determined from the slope of the linear portion of a semilogarithmic <sup>59</sup>Fe retention plot (the natural logarithm of whole-body radioactivity, corrected for physical decay, vs time from days 11 to 35 of each diet period,  $t_{1/2B} = -\ln(2)/\text{slope}$ ).

#### Statistics

Results were evaluated by analysis of variance (ANOVA) for a crossover design (15). Variation in the data was expressed as a pooled SD, calculated as the square root of the mean square error from the ANOVA.

#### Results

After an equilibration of > 3 wk, measurements of fecal iron and apparent iron absorption for 9 d were not improved by use of a visual fecal marker. Results from a predetermined sampling schedule and a fecal marker were similar in magnitude and variability and were highly correlated ( $r^2 = 0.80$ , *P* < 0.0001). Consequently, only results from the prescheduled collection method are presented.

Ascorbic acid supplementation did not affect apparent iron absorption by women with low iron stores whether they consumed a diet with predicted poorly bioavailable iron (ascorbate  $5.8 \pm 3.1$  vs placebo  $4.3 \pm 2.0$  mg/d, NS) or a typical Western diet ( $6.0 \pm 4.5$  vs  $6.4 \pm 2.1$  mg/d, NS) (Table 2). Ascorbic acid supple-

mentation had no consistent effect on apparent iron absorption by individuals. Apparent iron absorption did not correlate with serum ferritin, with or without logarithmic transformation, within this narrow range of low serum ferritin values.

Ascorbic acid did not affect most biochemical indexes of iron or thyroid status (Table 2). Two exceptions were higher total  $T_3$  in experiment 1 ( $1.9 \pm 0.4$  vs  $1.6 \pm 0.3$  nmol/L,  $P < 0.02$ ) and higher serum iron in experiment 2 ( $16.2 \pm 3.4$  vs  $15.3 \pm 3.7$   $\mu$ mol/L,  $P < 0.004$ ), but these findings did not persist when data from both experiments were combined (Table 2). Combined data suggested slightly higher ferritin with ascorbic acid ( $11.9 \pm 5.2$  vs  $10.7 \pm 3.8$ ,  $P < 0.06$ ). Ascorbic acid did not affect  $^{59}\text{Fe}$  biological half-life in experiment 1 (Table 2); this was not tested in experiment 2.

## Discussion

Most studies of the enhancement of iron absorption by ascorbic acid have measured erythrocyte incorporation of iron radioisotope from single meals fed 2 wk earlier. By this method, ascorbic acid, either synthetic or from foods (1), has consistently and substantially enhanced nonheme-iron absorption. Enhancement was observed whether addition of radioisotope was intrinsic during growth of the food (16), or extrinsic in the final stages of food preparation (1, 2, 17). Enhancement was also observed by monitoring fecal excretion of stable isotopes (18), and by elemental balance in the study of women recovering from phlebotomy (6).

In 1978, Monsen et al (8) published a model to estimate the bioavailability of iron in a meal based on the meat and ascorbic acid content. This model estimates a fourfold increase in absorption of nonheme iron by persons with low iron stores if ascorbic acid is increased to  $\geq 75$  mg in meals otherwise low in ascorbic acid and meat (8). If this predictive model were applied to the present study of women with low iron stores, true iron absorption from the diet with low predicted iron bioavailability (experiment 1) would have increased from 5% to 20%, a difference of  $\approx 2.5$  mg/d. Less ascorbic acid enhancement of iron absorption might be expected from the typical Western diet (experiment 2), because of higher basal amounts of enhancing factors (meat and ascorbic acid) and a greater proportion of iron in the heme form. The model considers the effect of ascorbic acid from 0 to  $\geq 75$  mg/meal (8). This could be expected to underestimate enhancement of iron absorption for the present experiments with 500 mg ascorbic acid/meal, because enhancement has been found to increase logarithmically with as much as 1000 mg ascorbic acid/meal (1, 17). Addition of 500 mg ascorbic acid to single meals increased nonheme-iron absorption by 2.3-fold with a breakfast meal; 3.5-fold with a hamburger meal; 6.5-fold with a Latin American meal of maize, beans, and rice (1); and sixfold with a semisynthetic meal (17). However, the present study does not confirm a substantial enhancement of iron absorption by ascorbic acid.

Why do the present results appear incompatible with predictions derived from  $^{59}\text{Fe}$  labeled meals? Reasons could include insubstantial statistical power to determine differences in apparent absorption (metabolic balance method), insensitivity of serum ferritin to modest improvements in iron status, and/or an overestimation of the enhancing effects of ascorbic acid derived from previous results with single labeled meals. These will be discussed further below.


The high variability of fecal excretion and apparent absorption measurements reduces the statistical power to detect differences with the small sample sizes used. The variability of these measurements in the present experiments (pooled SD  $\approx 2.5$ , Table 2) allowed 60–70% power ( $\alpha = 0.05$ ) to detect a difference of 2.5 mg Fe in experiments with 12–13 people, and 90–95% power to detect such a difference with the two experiments combined ( $n = 25$ ) (19). There was very little statistical power to distinguish the smaller differences in apparent absorption actually observed from random error. In a previous experiment with methods and a diet similar to our experiment 1, but with 11 women recovering from iron depletion by phlebotomy (6), ascorbic acid significantly improved apparent iron absorption (6.3 vs 4.0 mg/d, pooled SD 1.8), hemoglobin, zinc protoporphyrin, and serum iron (but not serum ferritin). The highly positive apparent iron absorption in both the present study and the previous study (6) may relate to menstrual iron losses and possibly to more dietary iron in the experimental diet than the volunteers commonly consumed.

In contrast to apparent absorption measurements, the variability in serum ferritin measurements was small enough to allow detection of modest differences associated with ascorbic acid. The lack of effect of ascorbic acid on serum ferritin in the previous study of iron-depleted women (6) was attributed to use of the additional absorbed iron for hemoglobin repletion (20). In the present investigation, the participants' hemoglobin concentrations ranged from 113 to 147 g/L and transferrin saturation values ranged from 15% to 36%; both were unaffected by ascorbic acid so enhanced iron absorption could be expected to affect iron stores as indicated by serum ferritin. Enhancement of iron absorption by 1.5 (observed difference, experiment 1, Table 2, NS) or 2.5 mg/d (the predicted difference) for 5 wk could be expected to increase serum ferritin values by 5 or 10  $\mu$ g/L, respectively, according to the general guideline that 120  $\mu$ g/kg storage iron corresponds to 1  $\mu$ g/L serum ferritin (21), and adjusting for iron lost in blood samples. The observed difference in serum ferritin of 1.2  $\mu$ g/L (10.7 with placebo vs 11.9 with ascorbate,  $n = 25$ ; Table 2) would correspond to an increase in iron absorption of  $\approx 0.3$  mg/d, by using the same criteria. The lack of a substantial response of serum ferritin to ascorbic acid in the present study, and in studies of ascorbic acid supplementation of persons with self-selected diets and varied iron stores (3–5), suggests that ascorbic acid supplementation has a more modest effect on iron absorption than is indicated by studies with single meals, and/or that serum ferritin is not a good indicator of short-term improvements in iron stores. Iron supplementation has been associated with modest increases in serum ferritin. Serum ferritin values increased significantly from  $14 \pm 9$  to  $26 \pm 13$   $\mu$ g/L in 16 women selected for low serum ferritin who were supplemented with 120 mg Fe/d for 7 wk (without a placebo comparison; 22); this suggests  $\approx 2\%$  absorption of a high-dosage supplement. In another study of women with low serum ferritin values without anemia, iron supplements of 18–20 mg as either extracts of herbs and yeast or as iron fumarate with 120 mg ascorbic acid taken with breakfast for 6 mo, increased serum ferritin values by 4.9  $\mu$ g/L ( $P < 0.01$ ) or by 3.9  $\mu$ g/L (NS), respectively (without a placebo control), suggesting  $< 1\%$  absorption of a supplement given with a meal (23).

The enhancing effects of ascorbic acid may have been overestimated from results with single labeled meals. Tests with sin-



gle labeled meals have usually been conducted in the morning after an overnight fast. The majority of dietary iron is consumed later in the day (17) when the enhancing effect of ascorbic acid may be dampened by the presence of residual food in the stomach or small intestine. Cook et al (24) reported less influence of both dietary enhancers and inhibitors of nonheme-iron absorption from whole diets (28 meals—the two largest meals of each day—labeled in a 2-wk period) than from single meals tested in the morning in the same individuals. The iron bioavailability model predicts that  $\geq 75$  mg ascorbic acid will increase iron absorption fourfold (from  $\approx 5\%$  to  $20\%$ ) in women with low iron stores and twofold (from  $\approx 2\%$  to  $4\%$ ) in men with an average of 1000 mg storage iron (8). However, we have been unable to identify experimental evidence that relates iron status to the degree of enhancement of iron absorption by ascorbic acid. In data presented by Cook and Monsen (17), we found no correlation between serum ferritin and the ratio of iron absorption observed with to that without ascorbic acid supplements of 100 mg ( $n = 25$ ) or 500 mg ( $n = 25$ ). In tests with single meals, the enhancing effects of ascorbic acid depend on the characteristics of the meal. Hallberg et al (1) reported less enhancement by ascorbic acid given with breakfast meals or hamburger meals, and possibly less with a Thai rice meal than with a Latin American maize, beans, and rice meal; a pizza meal; or a semisynthetic meal. The enhancement of iron absorption by 100 mg ascorbic acid was approximately fourfold with a semisynthetic meal containing dextrimaltose, corn oil, and ovalbumin, and 1.6-fold with a meal of beef, potatoes, corn meal, peaches, ice milk, bread, and margarine (17). Adjustment of the bioavailability model to a 1.5- to twofold enhancement of iron absorption by ascorbic acid, independent of iron status, may better describe the influence of ascorbic acid on iron absorption from Western diets.

It is not entirely clear why ascorbic acid substantially enhances iron absorption from single, radioisotope-labeled meals but does not affect clinical indicators of iron status. The present results question the predicted magnitude of ascorbic acid enhancement of iron bioavailability from Western diets for a population group with great potential benefit—women with low iron stores. The effects of ascorbic acid on body iron retention from whole diets seem to be considerably more modest and gradual than effects predicted from studies with single meals. 

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